Fall 2014

Synthesis Of Multilayered Microparticles For Targeted Drug Delivery

Elizabeth Mercer
Purdue University

Graduate School Form 30
(Revised 08/14)

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Elizabeth Mercer

Entitled

Synthesis of Multilayered Microparticles for Targeted Drug Delivery

For the degree of Master of Science in Biomedical Engineering

Is approved by the final examining committee:

Alyssa Panitch

James Leary

Kinam Park

To the best of my knowledge and as understood by the student in the Thesis/Dissertation Agreement, Publication Delay, and Certification/Disclaimer (Graduate School Form 32), this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Alyssa Panitch

Approved by Major Professor(s): _____

_____

Approved by: George Wodicka 12/01/2014

Head of the Department Graduate Program Date

SYNTHESIS OF MULTILAYERED MICROPARTICLES FOR TARGETED DRUG DELIVERY

A Thesis

Submitted to the Faculty

of

Purdue University

by

Elizabeth Mercer

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science in Biomedical Engineering

December 2014

Purdue University

West Lafayette, Indiana

For Dhruv Garg

ACKNOWLEDGEMENTS

I would like to begin by thanking all my family and friends for their support throughout this process. My mom, Katherine Armstrong and dad, Brian Mercer have truly supported me throughout all my endeavors. The love and support of Walter and Daphne Mercer have also been essential through this process. I would like to Becky, Jim, and Dexter Lesh for all the good times and support that they have given me.

I would like to thank all the Panitch lab members for all the good times and collaboration. Specifically, Jim McMasters who was always there to answer my questions and gave me good suggestions throughout my experiments. Also, I would like to thank Scott Poh for all his help with teaching me to use various imaging devices. I am excited to see all the future great works of the Panitch lab members. I would like to thank to everyone in the Weldon School of Biomedical Engineering and Purdue University for shaping a wonderful undergraduate and graduate career

Finally, I am most grateful for all my committee members: Dr. Alyssa Panitch, Dr. James Leary, and Dr, Kinam Park for their invaluable input and suggestions to future this work. But truly all this would not have been possible without my major professor, Dr. Panitch, who is brilliant and provided great insight and suggestions to my project. I am truly grateful for her allowing me to be a part of her lab and for all her guidance.

TABLE OF CONTENTS

Page

ABSTRACT

Mercer, Elizabeth. M.S.B.M.E., Purdue University, December 2014. Synthesis of Multilayered Microparticles for Targeted Drug Delivery. Major Professor: Alyssa Panitch.

Microparticles have been shown to be valuable in targeted drug delivery which can lead to an increased dose delivered to a targeted location, reduced patient side effects, and improved patient outcomes. The designed multilayered microparticles have the clinical application to deliver hydrophobic drugs to a targeted area. The composition of the microparticles consists of a poly-lactic acid (PLA) polymer core surrounded by a polymeric shell composed of Poly(lactic-co-glycolic acid)-Poly(ethylene glycol)-Maleimide(PLGA-PEG-Mal). The maleimide promotes conjugation of the collagen binding peptide, SILY. Targeting to type I collagen allows for this microparticle system to attach to exposed collagen in atherosclerotic vessels.

A novel templating method was used to synthesize uniform microparticles with manufacturing scale-up potential. Modification of the templating method was necessary to synthesize hydrophilic, multi-layered microparticles. The experimental results verified and quantified the presence of attached fluorescent SILY to the maleimides present on the surface of the microparticles as well as the microparticles' attachment to type I collagen. These results signify that the designed microparticles have the potential to deliver hydrophobic drugs to type I collagen throughout the body and potentially target atherosclerotic regions.

CHAPTER 1. INTRODUCTION

1.1 Drug Delivery Systems

Drug delivery systems advancements have led to the treatment of a variety of diseases. Drug delivery systems are defined as "engineering technologies for the targeted delivery and/or controlled release of therapeutic agents" [1]. Nanoparticles and microparticles are growing as a major component of novel drug delivery processes. Nano and microparticles have been shown to be valuable in the following:

- targeted drug delivery to diseased tissues
- decreased dose delivered systemically and potential tissue damage [2]
- increased dose delivered locally to diseased tissues
- reduced side effects in sensitive, non-target tissues [2]
- controlled drug release [3]
- improved patient outcomes

Ultimately, nano and microparticles have the potential to provide improved therapy and diagnostics to more patients.

Due to the increased amount of research performed on nano systems, it is important to understand the distinction between nano and microparticles. Nano and microparticles are so referred based on the distinction between dimensions in terms of nanometer and micrometers respectively; these size differences can have numerous effects on the mode and type of therapy delivered[4]. The benefits of larger particles are as follows:

- they are less likely to aggregate and potentially cause occlusions [4]
- they have the potential for higher drug loading
- they have a longer time for degradation

Microparticles have a tendency to remain localized in diseased tissue after direct injection [4]. For example, microparticles with 5-250 μm diameter remained in the peritoneum of mice for at least two weeks compared to the same mass of nanoparticles which were cleared from the peritoneum in two days [4, 5]. Microparticles are the chosen drug delivery systems in order to provide increased long-term treatment to diseased areas.

Similar to any system, microparticles have their limitations. Smaller particles have better binding affinity compared to larger particles [4]. The binding affinity can be combated by adding a targeting ligand, such as a peptide, to the outside of the microparticle to increase binding to particular cells. Also, larger microparticles have the potential to cause embolization within blood vessels. They may lodge in the pulmonary vasculature causing a pulmonary embolism or may lodge in end organs causing ischemia and possibly necrosis. The exact particle size to prevent this is unknown, but large quantities of 4-5μm particles were injected into the carotid arties of mice without causing detectable problems [4, 6]. Based on previous research, 5μm is the upper limit for target microparticle size to prevent embolization potential. Due to the numerous benefits of microparticles as well as the methods to combats their limitation, microparticles are the chosen drug delivery system.

1.2 Microparticle Design

Microparticles can be adaptable with the delivery of different drugs to various locations within the body based on their unique design. The overall design of the

microparticles in this study consisted of a hydrophobic polymer core surrounded by a hydrophilic polymer shell with attached targeting peptides shown here in Figure 1.1.

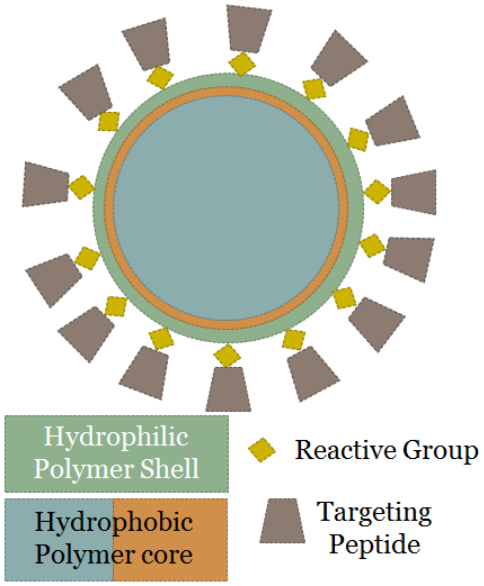

Figure 1.1 Depiction of the overall designed microparticle system. The blue and orange regions represent the hydrophobic core surrounded by the green hydrophilic shell. The polymer shell also contains a reactive group which allows for the attachment of the targeting peptide.

This amphiphilic system provides many advantages to this drug delivery system by having the potential to incorporate a hydrophobic drug within the hydrophobic polymer core. Hydrophobic drugs tend to have poor solubility in the body's aqueous environment [2]. The amphiphilic system enhances drug solubility by providing both a hydrophilic environment for the body to interact with and a hydrophobic environment to encapsulate the drug [2].

1.2.1 Hydrophobic Polymer Core

The hydrophobic polymer core is composed of poly-lactic acid (PLA). PLA is commonly used for controlled drug delivery because it is a well-known, biodegradable, non-toxic polymer [7,8]. PLA was chosen as the core material due to its hydrophobic

properties, which inhibited diffusion into the hydrophilic PVA template and provided the structural integrity for the microparticles.

The hydrophobic effect of the PLA core promotes interaction with the hydrophobic region of the polymer shell. Poly(lactic-co-glycolic acid)- Poly(ethylene glycol)- Maleimide (PLGA-PEG-Mal) composes the polymer shell, however the hydrophobic PLGA incorporates into the core with which it is miscible. PLGA provides many benefits in medical systems due to its biodegradability, biocompatibility, predictability of degradation, ease of fabrication, and FDA approval [9, 10].

1.2.2 Hydrophilic Polymer Shell

Polymer shell coatings provide many advantages to drug delivery systems. A significant advantage is that these shells provide drug diffusion control which slows the release of drug from the core of the microparticle [3]. The polymer used to form the shell is Poly(lactic-co-glycolic acid)-Poly(ethylene glycol)–Maleimide (PLGA-PEG-Mal). While the PLGA intercalates into the core to hold the shell in place, the PEG remains largely within PEG-rich layer at the particle surface. Significant research has been performed on the use of PLGA and PEG in drug delivery systems. These polymers are both biodegradable and their rate of degradation is linked to the chain length [11]. PLGA-PEG microparticles have been synthesized to deliver pulmonary drugs [12], anti-inflammatory drugs [13], and neurotrophic factor used to treat Parkinson's Disease [14]. Based on the numerous benefits of PLGA and PEG, these polymers are ideal for use as a polymeric shell for this microparticle system.

Since the hydrophobic PLGA incorporated into the core, the hydrophilic PEG is the main component of the shell. The PEG facilitates the aqueous-based conjugations and

inhibits particle aggregation within the body [15]. PEG coatings have been found to be non-immunogenic, so they provide protection against interaction with the blood components, which induce the removal of the foreign particles from the blood [16, 11]. Therefore, the circulation time of microparticles with PEG ensures that the microparticles have sufficient time to target and deliver therapy to the diseased areas within the blood vessels.

The PEG has a terminal maleimide functional group allowing for attachment of targeting peptides containing a sulfhydryl or amine. The PEG is highly flexible which allows for peptide conjugation to the maliemide without significant steric hindrance of the peptide ensuring that it is available to bind to its target [16]. For reaction mixtures with a pH greater than 8.5, the maleimide favors a reaction with a primary amine, but also the alkaline pH increases the rate of hydrolysis to a non-reactive maleamic acid [17]. For reaction mixtures with a pH between 6.5 and 7.5, the maleimide reacts with a sulfhydral to form a stable, nonreversible thioether linkage [17]. All experiments were executed at a pH of 7.4 to enhance maleimide reaction with sulfhydryl groups. Buffers or agents with free sulfhydryls or free amines should not be used in the synthesis of these microparticles as they have the potential to react with the free maleimides. This overall microparticle design has the potential to be used to deliver various hydrophobic drugs to various targeted tissues within the body based on the chosen targeting peptide.

1.3 Microparticle Application

1.3.1 Atherosclerosis Background

Atherosclerosis is most commonly characterized by the accumulation of lipids and fibrous elements in the large arteries; however, it is now recognized as a chronic inflammatory disease. [18, 19]. Atherosclerosis has the clinical implication of a build-up of plaque along artery walls which potentially leads to heart attack, stroke, or even death [18, 20]. Collagen Type I is a major component of deep vessel injury on an atherosclerotic vessel injury present after balloon angioplasty or plaque rupture [21]. The target diseased area for these particular microparticles is atherosclerotic tissue along vessel walls.

1.3.2 Collagen Binding Peptide

Collagen is the most abundant protein in animals [23]. It is located in and maintains the integrity of the extracellular matrix and connective tissue [23, 24]. Due to the mechanical resilience of collagen, it has become highly used for different biomedical applications including: biomimetic cell culture scaffold and tissue engineering [25, 26]. There are at least twenty-nine types of collagen, but types I, II, and III are the most abundant [27]. The building block of collagen is tropocollagen which is composed of three helical polypeptide chains that containing the triplet Gly-X-Y [27, 23]. Type I collagen has many benefits including its ability to self-assemble *in vitro*, resilient mechanical properties, and hierarchical structure [26]. The microparticles will be tested to see if attachment occurs with type I collagen from rat tail [28]. Rat tail tendon-rich tissue makes fibrous type I collagen easy to isolate and readily available [24]. The

fundamental collagen unit of rat tail type I collagen is a long, thin protein that consists of three coiled subunits [24]. Due to the abundance of collagen in the body, there are many diseases associated with collagen that would benefit from a drug delivery system targeting collagen. For this reason, a collagen binding peptide was then attached to the polymer shell to promote collagen type I attachment.

In order to effectively target type I collagen, a collagen binding peptide can be attached to the shell of the microparticles. Various synthetic peptidoglycans that bind to type I collagen have been or are currently being synthesized in the Panitch laboratory [29-31]. During development of the peptidoglycans, the peptide sequence RRANAALKAGELYKSILYGC (SILY) was shown to bind with Collagen Type I on its own or when bound to hydrophilic polymers [31]. SILY was derived from the platelet receptor to collagen type I and was modified to use a cysteine sulfhydryl group for conjugation to polymers [31]. The sulfhydryl group at the C-terminus of SILY allows for conjugation to the maleimides available on the shell of the microparticles. For this reason, SILY was the chosen collagen-binding peptide for this system.

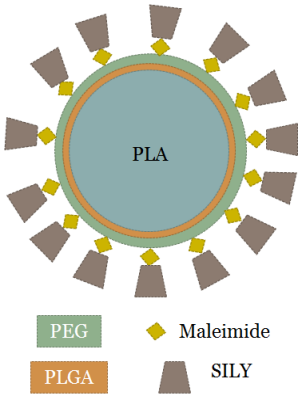

Figure 1.2 Depiction of the overall microparticle system including the collagen binding peptide, SILY described below.

1.4 Microparticle Synthesis Method

1.4.1 Common Synthesis Methods

To date, microparticles have been prepared by emulsion-solvent evaporation, phase separation, interfacial polymerization, spray drying, emulsion extraction process, jet milling technique, and fluidization [10]. The common emulsion methods result in particles with different size distributions [32]. The uniformity of the size of the particles has a significant influence on the drug release rates. Particle size can be controlled by various factors for the conventional synthesis methods including: varying polymer concentration [33] and choice of polymer chemistry [34]. Various studies have found difficulties in producing uniformly sized particles for drug release studies [33, 35]. However, some researchers have shown that larger particles release encapsulated compounds more slowly and over an extended time period with the other properties (molecular weight, initial porosity, drug distribution with the sphere) being equal [34]. Drug release studies have shown that the initial drug release rates decreased with increasing particle diameter due to the decrease in surface/volume ratio with increasing size [34]. This resulted in a concave downward and sigmoidal release profile curve for small and large particles respectively [34]. Thus, the uniformity of microparticles is essential to understanding and controling drug release rate profiles. Fortunately, a templating method developed by Dr. Kinam Park's lab at Purdue University has shown promise in producing uniformly sized particles among other benefits.

1.4.2 Park Templating Method

The particle size can be easily controlled using the Park templating method just with the use of different template sizes ranging from 6-50μm [36]. The template used in these studies made 50μm column height and diameter microparticles. The Park templating method

also has the scale-up potential for manufacturing using the new automated microparticle synthesizer, the SpinSwiper [36].

The Park templating method (shown in Figure 1.3) produces microparticles by using a silicon master template. The silicon master template is then used to form a degradable template made of gelatin or poly(vinyl alcohol) (PVA). The desired polymer solution is poured into the gelatin or PVA template with the excess solution removed. The solvent in the polymer solution evaporates, and the gelatin or PVA can be melted and removed leaving column shaped particles. Since the shape of the particles has previously been limited to a sphere, the Park templating method will allow research of different particle shapes on biological responses. Another benefit of this synthesis method is that the gelatin mold dissolved so the particles are released without mechanical methods.

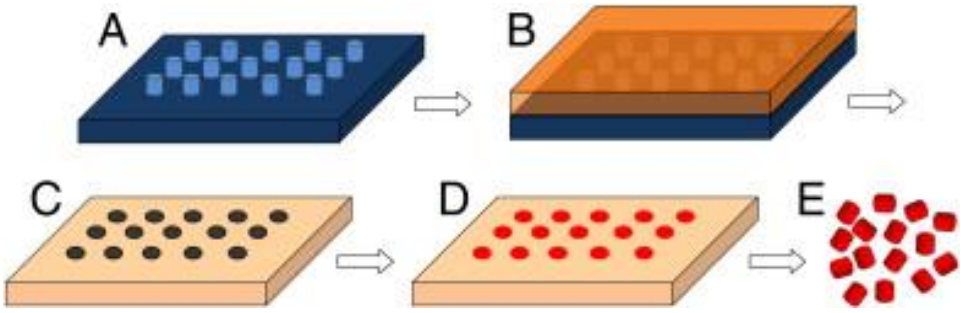

Figure 1.3 Schematic of the Park Microparticle Synthesis Process [32]. A: The silicon master template is made by photoliography. B: A warmed gelatin solution is cooled on the master template. C: The master template is peeled away exposed open wells to make uniform microparticles. D: The wells are filled with polymer solution and the excess solution is removed with a razor blade. E: The gelatin is heated and removed with centrifugation. And, the final product of the microparticles remains.

In order to adapt this procedure to synthesize the multilayered microparticles, the PLGA-PEG-MAL and PLA are added in separate layers. The hypothesis with this procedure was that the hydrophilic PEG would preferentially face or diffuse into the template surface.

And, the hydrophobic PLGA would preferentially remained exposed to the air interface and would be available to intercalate into the core of the particles when the PLA solution was added to the template. The final PEG-PLGA layer to the template particle surface would allow for complete coating of the PLA particles with a PEG shell. Adapting the Park templating method for synthesizing multilayered microparticles could be used to treat a variety of diseases in various locations of the body.

The overall goal of this research is to develop a novel microparticle drug delivery system synthesized by adapting the Park templating method. Chapter 2 describes the adapted synthesis method and the methods for analyzing the synthesized microparticles. Chapter 3 shows the obtained results of verifying surface maleimides and the collagen binding capabilities of the microparticles. Chapter 4 describes the benefits of this microparticle system as a novel platform for new drug delivery methods.

1.5 References

[1] Drug Delivery Systems: Getting Drugs to Their Targets in a Controlled Manner. `*National Institute of Biomedical Imaging and Bioengineering,* 2014.

[2] Allen, T.M. and Cullis, P.R., Drug delivery Systems: Entering the Mainstream. *Science,* 2004. **303**(5665): p. 1818-1822.

[3] Langer, R.S. and Peppas, N.A., Present and Future Applications of Biomaterials in Controlled Drug Delivery Systems. *Biomaterials,* 1981. **2**(4) p. 201-214.

[4] Kohane D.S., Microparticles and Nanoparticles for Drug Delivery. *Biotechnology and Bioengineering*, 2007. **96**(2): p. 203-209.

[5] Kohane, D.S., Tse, J.Y., Yeo, Y., Padera, R., Shubina, M. and Langer, R., Biodegradable Polymeric Microspheres and Nanospheres for Drug Delivery in the Peritoneum. *Journal of Biomedical Materials Research Part A,* 2006. **77**(2): p. 351-361.

[6] Kohane, D.S., Plesnila, N., Thomas, S.S., Le, D., Langer, R., and Moskowitz, M.A., Lipid–Sugar Particles for Intracranial Drug Delivery: Safety and Biocompatibility. *Brain Research,* 2002. **946**(2): p. 206-213.

[7] Jamshidian, M., Tehrany, E. A., Imran, M, Jacquot, M., and Desobry, S. Poly-Lactic Acid: Production, Applications, Nanocomposities, and Release Studies. Comprehensive Reviews in Food Science and Food Safety. *Comprehensive Reviews in Food Science and Food Safety,* 2010. **9**(5): p. 552-571.

[8] Lassalle, V. and Ferreira, M.L., PLA Nano- and Microparticles for Drug Delivery: An Overview of the Methods of Preparation. *Macromolecular Bioscience,* 2007. **7**(6): p. 767-783.

[9] Danhier, F., Ansorena, E., Silva, J.M., Coco, R., Le Breton, A., and Preat, V., PLGA-based Nanoparticles: An Overview of Biomedical Applications. Journal of Controlled Release. *Journal of Controlled Release,* 2012. **161**(2): p. 505-552.

[10] Satheesh Madhav, N.V. and Kala, S. Review on Microparticulate Drug Delivery System. *International Journal of PharmTech Research,* 2011. **3**(3): p. 1242-1254.

[11] Ravi Kumar, M.N.V., Nano and Microparticles as Controlled Drug Delivery Devices. *Journal of Pharmaceutical Sciences*, 2000. **3**(2): 234-258.

[12] Takami, T. and Murakami, Y., Development of PEG–PLA/PLGA Microparticles for Pulmonary Drug Delivery Prepared by a Novel Emulsification Technique Assisted with Amphiphilic Block Copolymers. *Colloids and Surfaces B:Biointerfaces*, 2011. **87**(2) 433-438.

[13] Gaignaux, A., Reeff, J., Siepmann, F., Siepmann, J., De Vriese, C., Goole, J., and Amighi, K., Development and Evaluation of Sustained-Release Cloidine-Loaded PLGA Microparticles. *International Journal of Pharmaceutics,* 2012. **437**(1-2): p. 20-28.

[14] Garbayo, E., Ansorena, E., Lanciego, J.L., Aymerich, M.S., and Blanco-Prieto, M.J., Sustained Release of Bioactive Glycosylated Glial Cell-line Derived Neurotropic Factor from Biodegradable Polymeric Microspheres. *European Journal of Pharmaceutics and Biopharmaceutics,* 2008. **69**(3): p. 844-851.

[15] TechNote 202, Microsphere Aggregation. *Bangs Laboratories, Inc.,* 2013.

[16]Hayworth, D., Polyethylene Glycol (PEG) and Pegylation of Proteins. *ThermoScientific*.

[17] Hayworth, D., Sulfhydryl-reactive Crosslinker Chemistry. *ThermoScientific*, 2013.

[18] Hansson, G.K., and Libby, P., The immune response in Atherosclerosis: A Double-Edged Sword. *Nature Reviews Immunology,* 2006. **6**: 508-519.

[19] Tang, L., Sakai, Y., Ueda, Y., and Katsuda, S., Effects of Oral Administration of Tripeptides Derived from Type I Collagen on Atherosclerosis Development in Hypercholesterolemic Rabbits. *Journal of Bioscience and Bioengineering,* 2014. p. 1-6.

[20] What is Atherosclerosis? *National Heart, Lung, and Blood Institute*, 2011.

[21] Badimon, L., Badimon, J.J., Turitto, V.T., Vallabhajosula, S. and Fuster, V., Platelet Thrombus Formation on Collagen Type I. A Model of Deep Vessel Injury. Influence of Blood Rheology, Von Willebrand factor, and Blood Coagulation. *Circulation*, 1988. **78**(6): 1431-1442

[23] Su, H.N., Ran, L.Y., Chen, Z.H., Qin, Q.L., Shi, M., Song, X.Y., Chen, X.L., Zhang, Y.Z., and Xie, B.B., The Ultrastructure of Type I Collagen at nanoscale: Large or Small *D*-spacing Distribution. *Nanoscale,* 2014. **6**(8134).

[24] W.H. Freeman and Company, Collagen: The Fibrous Proteins of Matrix. *Molecular Cell Biology*, 2000. **4**(22.3).

[25] Freed, L.E., Guilak, F., Guo, X.E., Gray, M.L., Tranquillo, R., Holmes, J.W., Radisic, M., Sefton, M.V., Kaplan, D., Vunjak-Novakovic, G., Advanced Tools for Tissue Engineering: Scaffolds, Bioreactors, and Signaling. *Tissue Engineering,* 2006. **12**(12): 3285-3305.

[26] Stamov, D. R. and Pompe, T., Structure and Function of ECM-Inspired Composite Collagen Type I Scaffolds. *Soft Matter,* 2012. **8**(40) 10200-10212.

[27] Merrett, K., Ljunggren, M.K., Mondal, D., Griffith, M., Rafat, M., Collagen Type I: A Promising Scaffold Material for Tissue Engineering and Regenerative Medicine. p. 1-43.

[28] Corning BioCoat Collagen I Multiwell Plates. *Fisher Scientific,* 2014.

[29] Paderi, J.E., Sistiabudi, R., Ivanisevic, A., and Panitch, A., Collagen-Binding Peptidoglycans: A Biomimetic Approach to Modulate Collagen Fibrillogenesis for Tissue Engineering Applications. *Tissue Engineering Part A,* 2009. **15**(10): p. 2991-2999.

[30] Abd-Elgaliel, W.R. and Tung, C.H., Exploring the Structural Requirements of Collagen-Binding Peptides. *Biopolymers,* 2013. **100**(2): p. 167-173.

[31] Paderi, J.E., and Panitch, A., Design of a Synthetic Collagen-Binding Peptidoglycan that Modulates Collagen Fibrillogenesis. *Biomacromolecules,* 2008. **9**(9): p. 2562-2566.

[32] Acharya, G., Shin, C.S., McDermott, M., Himanshu, M., Park, H., Kwon, I.C., and Park, K., The Hydrogel Template Method for Fabrication of Homogeneous Nano/microparticles. *Journal of Controlled Release,* 2010. **141**(3): 314-319.

[33] Bezemer, J.M., Radersma, R., Grijpma, D.W., Dijkstra, P.J., Van Blitterswijk, C.A., and Feijen, J., Microsphere for Protein Delivery Prepared from Amphiphilic Multiblock Copolymers: 2. Modulation of release rate. *Journal of Controlled Release,* 2000. **67**(2-3): p. 249-260.

[34] Berkland, C., King, M., Cox, A., Kim, K., and Pack, D.W., Precise Control of PLG Microshphere Size Provides Enhanced Control of Drug Release. *Journal of Controlled Release,* 2002. **82**(1): p. 137-147.

[35] Narayani, R. and Panduranga Roa, K., Gelatin Microsphere Cocktails of Different Sizes for the Controlled Release of Anticancer Drugs. *International Journal of Pharmaceutics,* 1996. **143**(2) 255-258.

[36] SpinSwiper. Akina, Inc. Available at: SpinSwiper.com.

CHAPTER 2. METHODS

2.1 PVA Template Synthesis

Poly(vinyl alcohol) (PVA) was purchased from Sigma-Aldrich. The 4% (w/v) PVA was dissolved in 500mL of deionized water with constant stirring at 60˚C. After full dissolving of the PVA, 500mL of ethanol was slowly added to the PVA solution with constant stirring at 60˚C. The PVA solution was left at room temperature.

2.1.1 Preparation of PVA Templates

A Polydimethylsiloxane (PDMS) template with 50µm column height and width wells was purchased from Akina, Inc (Cat # AKPDMS-50). The wells were formed with photolithography. The PDMS template was placed on a glass slide with the wells facing upright. Then, 8mL of PVA solution was pipetted onto the side of the PDMS template containing the wells. The PVA solution was allowed to dry at room temperature overnight on a flat surface. The dried PVA template was then peeled away from the PDMS template exposing the formed wells. The templates were stored in a dry, covered container until used. The PDMS molds were cleaned after approximately 30 pours with warm, soapy water. The PDMS molds were allowed to dry before the next pour.

2.2 Preparation of Polymer Solution

The acid terminated polymer, poly(DL-Lactide) (PLA), was purchased from Lactel Absorbable Polymers (CAT #B6014-1). The PLA has an inherent viscosity between 0.15-0.25kDa [1]. A 20% w/v solution of PLA in Dichloromethane (DCM) was prepared in a glass vial. And the solution was mixed for 30 minutes. This PLA solution was prepared to later form the core of the microparticles.

The Poly(lactide-co-glycolide)-b-poly(ethylene glycol)-maleimide (PLGA-PEG-Mal) was purchased from Akina, Inc. (Cat #AI20). The molecular weight of the PLGA-PEG-Mal was 20:5 kDa. The PLGA-PEG-Mal was prepared as a 5% solution in DCM. The polymer-solvent solution was mixed for 30 minutes. The PLGA-PEG-Mal was prepared to later form the shell of the functionalizable microparticle

The Methoxy poly(ethylene glycol)-b-poly(lactide-co-glycolide) (mPEG-PLGA) was purchased from Akina, Inc. (Cat #AK02). The molecular weight of the mPEG-PLGA was 2:4.11 kDa. The mPEG-PLGA was prepared as a 5% solution in DCM and was mixed for 30 minutes. The mPEG-PLGA was prepared to later be used as the shell of the negative control microparticles.

2.3 Microparticle Synthesis

The PVA templates were prepared for microparticle synthesis by cutting each PVA template into approximately 4 strips without any bubbles in the template. The templates were each placed onto an individual glass slide and taped on the top and bottom to prevent shifting.

2.3.1 Functionalizable Microparticle Synthesis [2]

The functionalizable microparticles were composed of a PLA core with a PLGA-PEG-Mal shell. These functionalizable microparticles were formed by pippetting 9µL of PLGA-PEG-Mal/DCM onto one PVA strip. The polymer solution was then quickly spread evenly around the PVA strip. This process was repeated for each strip. The polymer solution was allowed to dry for 15 minutes.

The PLA solution was used to form the core of the microparticles on top of the PLGA-PEG-Mal layer. After allowing the PLGA-PEG-Mal solvent to evaporate, 9µL of 20% PLA solution was pipetted onto one strip and spread evenly with a razor blade. This step was repeated for each strip. The PLA solution was allowed to dry for 15 minutes. Then, the final layer of the shell was applied by pippetting 9µL of the 5% PLGA-PEG-Mal solution onto a single PVA strip and spreading evenly with a razor blade. This was repeated for each strip and then the polymer solution was allowed to dry for 15 minutes.

After the drying process, the strips were cut using a razor blade to remove them from the glass slides. The strips were then placed into a 50mL beaker.

2.3.2 Negative Control Microparticle Synthesis [2]

The negative control particles were synthesized with the same steps of the functionalizable microparticles except in place of the PLGA-PEG-Mal, the mPEG-PLGA was used because of the unreactive methyl group on the end.

2.4 Attachment of Peptides

2.4.1 Attachment of FITC-SILY

The FITC-Ahx-RRANAALKAGELYKSILYGC (FITC-SILY) was purchased as a custom order from GenScript. A 2mg/mL solution of FITC-SILY with 1x Phosphate Buffered Saline (PBS) with a pH of 7.4 was prepared in the dark. This solution was mixed for one hour. The FITC-SILY solution was placed on top of the strips in the 50 mL beaker and was stirred for one hour at room temperature in the dark. This allowed the PVA template to fully dissolve and the FITC-SILY to conjugate to the available maleimide groups at the particle surface. The dissolved PVA and excess peptide were removed by centrifuging the sample at 5,000rpm for 5 minutes and discarding the supernatant. A wash using 1x PBS was repeated three times. The microparticles were then viewed under a light microscope to view the integrity of the particles and lyophilized overnight. The microparticles were stored at -20˚C. The attachment of FITC-SILY was essential for the later validation of FITC-SILY on the surface of the microparticles and for the quantification of FITC-SILY attachment.

2.4.2 Attachment of SILY

The RRANAALKAGELYKSILYGC (SILY) was purchased as a custom order from GenScript. The same procedure was used for the attachment of SILY that was described for the attachment of FITC-SILY except that the FITC-SILY was prepared at a concentration of 0.0156mg/mL in 1x PBS.

2.5 Validation Experiments

2.5.1 Validation of FITC –SILY Attachment to Microparticle Surface

The presence of attached FITC-SILY was validated using an FV1000/IX81 confocal microscope. Samples of the FITC-SILY-Mal-PEG-PLGA and the negative control microparticles were placed onto glass slides. These slide were analyzed used the 488 laser of the confocal microscope for review.

2.5.2 Quantification of FITC-SILY Attachment to Microparticles

The procedure for the attachment of FITC-SILY for this experiment differed slightly from the procedure described above because it was noted that there was some FITC-SILY non-specific interactions and peptide clumping. The only differences to the procedure described above are listed here. To prevent non-specific interaction of FITC-SILY with negative control microparticles, a methyl-PEG-PLGA with a molecular weight of 5,000:4,000 Da (Cat# AK30) was purchased from Akina Inc and used to form the polymer shell of the negative control microparticles. To reduce the clumping, a 0.0156mg/mL solution of FITC-SILY in 1x Phosphate Buffered Saline (PBS) with a pH of 7.4 was prepared in the dark with a small amount of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) [3] (Thermo Scientific (Product# 20490)). This solution was mixed for one hour. Then one wash was performed to remove any undissolved FITC-SILY by centrifuging at 5,000rpm for 5 minutes. The FITC-SILY solution was placed on top of the strips in the 50 mL beaker and was stirred for one hour at room temperature in the dark. This allowed the PVA template to fully dissolve and the FITC-SILY to conjugate to the available maleimide groups at the particle surface. The dissolved PVA

and excess peptide were removed by centrifuging the sample at 5,000rpm for 5 minutes and discarding the supernatant. A wash using 1x PBS was repeated three times. The microparticles were then viewed under a light microscope to view the integrity of the particles and lyophilized overnight.

The following day, 100µL of 1xPBS was placed into twelve wells of a black 96-well plate with a clear bottom. 0.06mg of experimental and negative control microparticles was measured and placed into individual wells in the dark. Six replicates were analyzed for the experimental and negative control groups. The 96-well plate was analyzed with a SPECTRAmax M5 Plate Reader with the following settings:

- Endpoint reading
- Fluorescence – bottom read
- Excitation: 495nm
- Emission: 519
- Automix: Once for 5 seconds before first read
- Calibrate: On
- PMT: Medium
- Settle Time: Off
- Column Priority
- C. Speed: Normal

The data was then analyzed based on the output from the M5.

2.5.3 Validation of Microparticles' Collagen Binding Capabilities

The ability for the SILY-Mal-PEG-PLGA microparticles to bind to collagen was validated using a collagen-binding assay. Collagen I 96 clear well plates were purchased from Fisher Scientific (Cat # 08-774-1). The collagen plate was blocked with 2% BSA to prevent non-specific binding for one hour at 37˚C. The plate was washed three times with 1xPBS. The SILY-Mal-PEG-PLGA and negative control microparticles (Me-PEG-PLGA) were separately suspended in 1x PBS at a pH of 7.4 at a concentration of 1.4mg/mL. Then, 100µL of microparticle solution was pipetted into each of 6 wells of the collagen plate. The 96-well plate was covered and incubated for 30 minutes at 37˚C. The wells were then washed three times with 1x PBS. The microparticles present in each well were imaged with a light microscope and manually counted.

2.6 References

[1] Inherent Viscosity versus Molecular Weight. *Lactel Absorbable Polymers*. Available at: http://www.absorbables.com/technical/inherent_viscosity.html.

[2] Acharya, G., Shin, C.S., McDermott, M., Himanshu, M., Park, H., Kwon, I.C., and Park, K., The Hydrogel Template Method for Fabrication of Homogeneous Nano/microparticles. *Journal of Controlled Release,* 2010. **141**(3): 314-319.

[3] TCEP-HCL. *ThermoScientific,* 2014. Available at: http://www.piercenet.com/product/tcep-hcl.

CHAPTER 3. RESULTS

3.1 Validation of FITC –SILY Attachment to Microparticle Surface

The described protocol was developed after first performing the published Park method to synthesize microparticles that are composed entirely of PLGA [3]. The results from this preliminary data showed that particles had a tendency to agglomerate in the water solvent most likely due to the hydrophobic nature of PLGA. This agglomeration made imaging and the size analysis of individual particles difficult. This agglomeration was not observed with the PLGA-PEG particles in water indicating a presence of PEG on surface that forms hydrogen bonds with the water thus preventing agglomeration of the particles. The dispersed particles can be seen clearly in Figure 3.1 shown below. The presence of the hydrophilic PEG on the surface also provides the opportunity to attach peptides, via a reactive PEG end group, to the particle surface to encourage particle binding to a target molecule.

The Mal-PEG-PLGA microparticles shown in Figures 3.2 and 3.3 below validate that the FITC-SILY is interacting with the surface of the particles. This interaction is most probably a reaction between the maleimide on the microparticle and the free cysteine in the SILY. Figures 3.2-3.3 show distinctive green fluorescent rings around a non-florescent polymer core. This same lack of fluorescence was seen in the negative control microparticles in Figure 3.4, suggesting that the peptide is able to conjugate to the maleimide on the particles coated with the PLGA-PEG-maleimide.

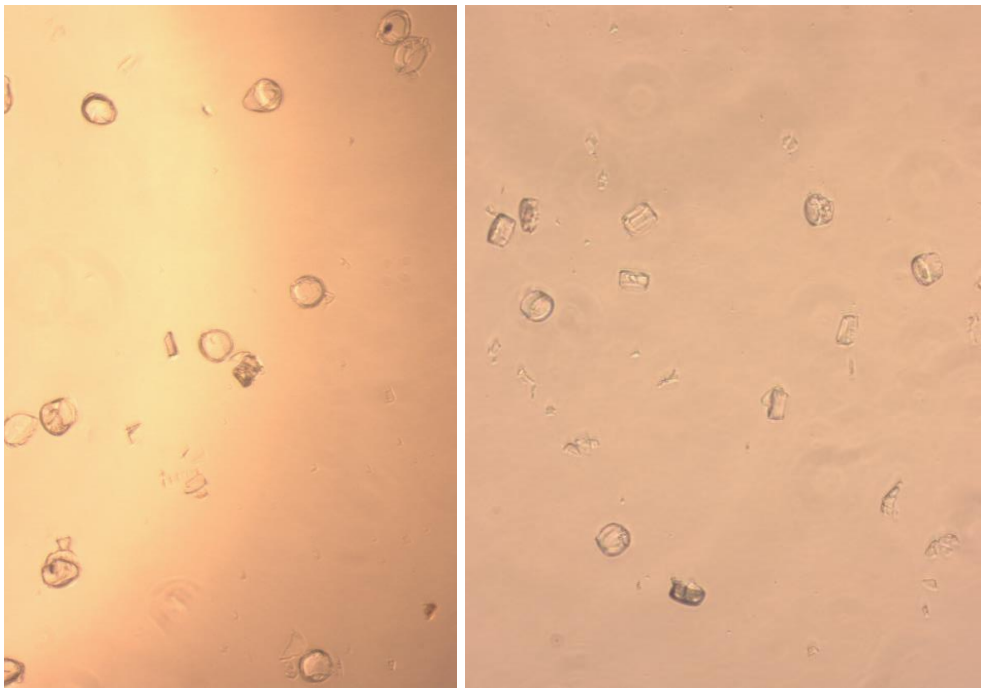

Figure 3.1 Light microscope images of the SILY-Mal-PEG-PLGA microparticles shown on the left. The right image shows the negative control microparticles composed of Me-PEG-PLGA.

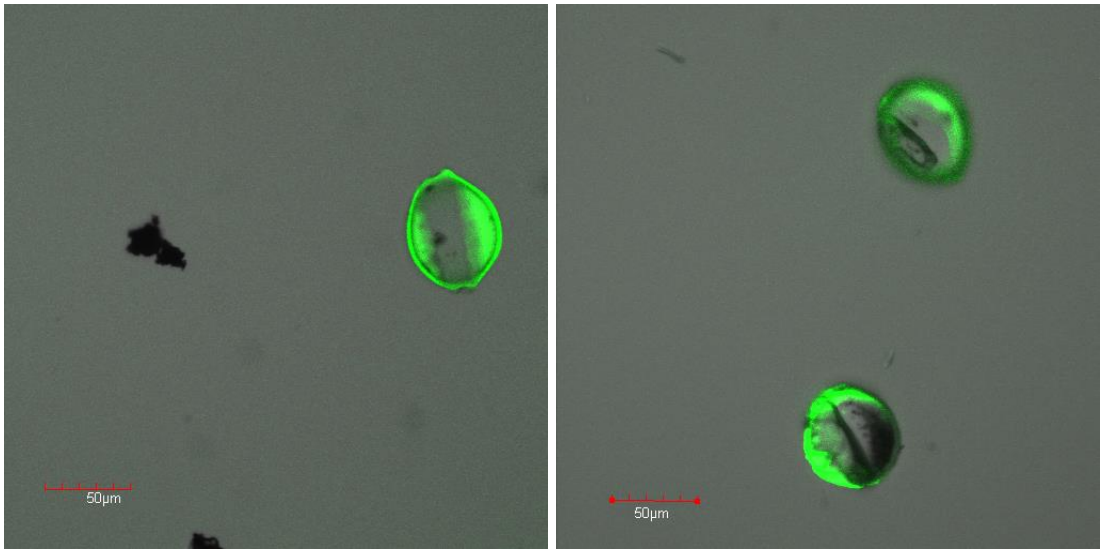

Figure 3.2 Mal-PEG-PLGA microparticles reacted with FITC-SILY. Microparticles were analyzed using a 488 laser on a confocal microscope. The microparticles are shown here in an overlayed image with white and fluorescent light. The microparticles shown in these two images represent microparticles in different batches on different days. This experiment was run in triplicate to ensure consistency of the results.

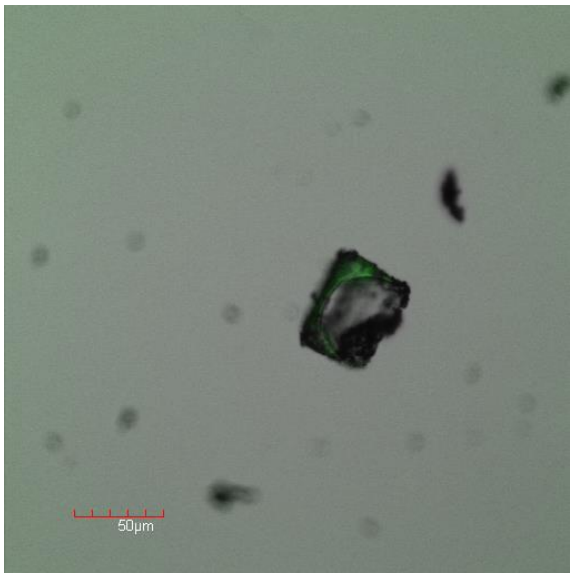

Figure 3.3 Negative control microparticles visualized with the confocal microscope with the 488 laser.

3.2 Quantification of FITC-SILY Attachment to Microparticles

3.2.1 Standard Curve

Table 3.1: Standard Curve Data of FITC-SILY Concentration from SPECTRAmax M5.

FITC-SILY Concentration	Fluorescence
0.015625	6638.9
0.0078125	2986.2
0.00390625	2071.6
0.001953125	767.98
0.000976563	924.43
0.000488281	164.89
0.000244141	107.81
0.00012207	92.616
6.10352E-05	91.295
3.05176E-05	58.673
1.52588E-05	60.723

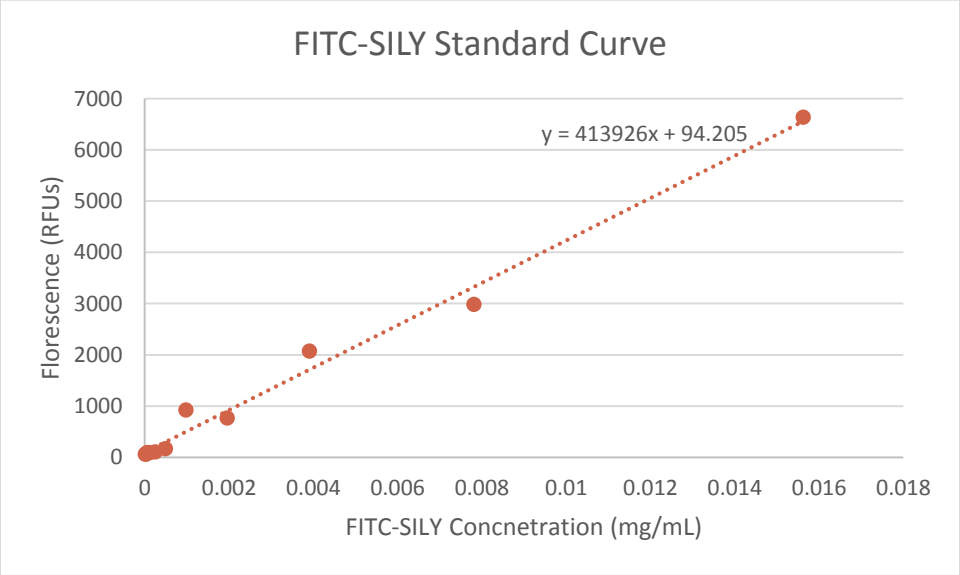

Figure 3.4: FITC-SILY Standard Curve graph used to quantify FITC-SILY attachment to microparticles.

3.2.2 Results of Quantification of FITC-SILY Attachment to Microparticles

Table 3.2: Experimental results showing the concentration of FITC-SILY Attached to experimental and negative control microparticles. Table Key: Exp= Experimental Samples, NC= Negative Control Samples, Avg=Average.

Exp	Sample	1	2	3	4	5	6	Avg	Standard Dev	Concentration of FITC-SILY (mg/mL)
1	Exp	716	473	285	682	3034	293	913.7	1054.8	0.00162737
	NC	162	209	171	269	178	180	194.8	39.6	0.000189537
2	Exp	580	223	497	306	166	420	365.2	161.0	0.00053036
	NC	133	106	109	205	96	95	124.0	42.1	4.79593E-05
3	Exp	474	126	324	264	231	88	251.2	139.8	0.0003024
	NC	40	54	49	55	58	77	55.6	12.4	-0.000088736
4	Exp	285	146	192	271	169	90	192.1	74.9	0.000184282
	NC	30	62	87	60	62	177	79.7	50.7	-4.05483E-05

The statistics were analyzed for the quantification experiment of FITC-SILY attachment to micorparticles. A normality test was first performed on each data group to determine normality. The normal data sets were analyzed using a two sample T-test, while the non-normal data was analyzed using a Mann-Whitney test.

Table 3.3: Statistical analysis showing a statistical difference between the experimental and negative control samples.

Experiment	Statistical Test	P-value	Alpha	Significantly Different
1	Mann-Whitney	0.0051	0.05	Yes
2	Mann-Whitney	0.0082	0.05	Yes
3	2 sample t-test	0.016	0.05	Yes
4	Mann-Whitney	0.0202	0.05	Yes

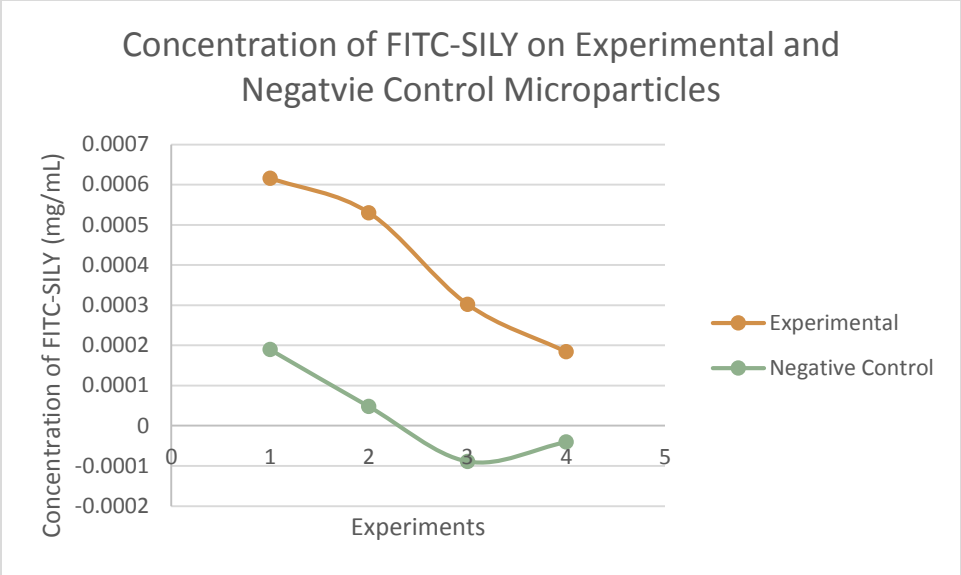

Figure 3.5: Graphical representation of the concentration of attached FITC-SILY to the experimental microparticles compared to the negative control microparticles.

Figure 3.5 does not include data point from Experiment 1 Sample 5 from the experimental microparticles because it is an outlier from this data. This outlier can be attributed to excess undissolved FITC-SILY present in the sample.

3.3 Results of Microparticles' Collagen Binding Capabilities

The ability of the attachment of a targeting peptide may greatly facilitate the application of the designed microparticles for drug delivery. SILY-Mal-PEG-PLGA microparticles were found to be attached to the collagen type I bound plate by counting whole microparticles attached to the collagen plate. Microparticle particulates were excluded from the microparticle count.

Table 3.4: Results of the attached microparticles to the collagen type I plate.

Experiment	Samples	1	2	3	4	5	6	Average	St Dev
1	Experimental	25	13	15	12	31	31	21.17	8.13
	Negative Control	2	1	1	5	6	5	3.33	2.05
2	Experimental	10	8	8	17	5	1	8.17	4.88
	Negative Control	1	4	3	1	1	1	1.83	1.21

A statistical analysis was performed on the collagen binding capability data. First, a normality test was run on all sets of data to determine if the data was normal. A two sample T-test was performed for normal data, and a Mann-Whitney test was performed for non-normal data.

Table 3.5: Statistical analysis of the attached microparticles to the type I collagen plate.

Test	P-value	Alpha	Significantly Different
2 Sample T-test	0.005	0.05	yes
Mann-Whitney	0.0247	0.05	yes

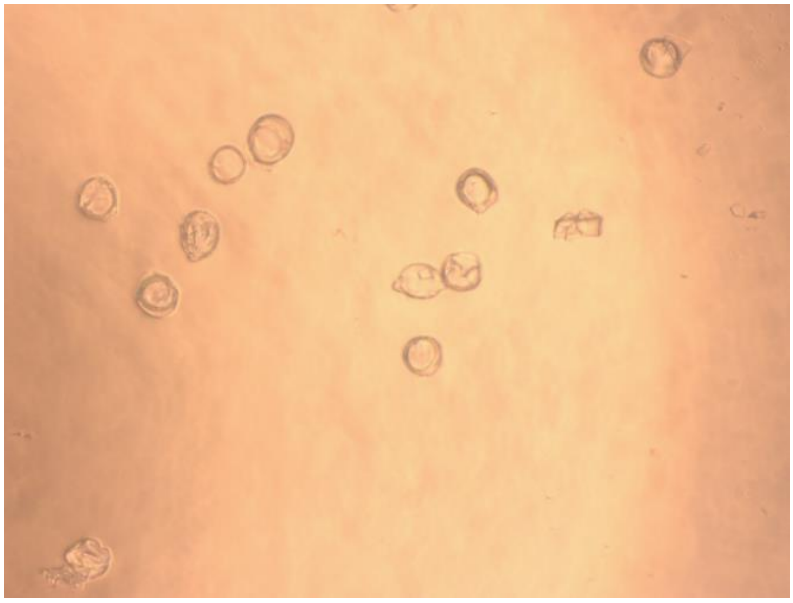

Figure 3.6: Bound experimental (SILY-Mal-PEG-PLGA) microparticles attached to the collagen type I plate. This image was taken from experiment 1's results.

CHAPTER 4. DISCUSSION

4.1 Adapting the Park Templating Method

4.1.1 Order of Assembly

There are many unique aspects of the Park templating synthesis method including the order of assembly. The designed microparticles are composed of an outer most layer of collagen binding peptides attached to a polymer shell. The polymer shell is composed of PLGA-PEG-Mal. The maleimides allow for the attachment of the collagen binding peptide. The polymer shell forms as an integrated layer with the hydrophobic PLA in the core of the microparticle and presents the hydrophilic PEG on the outside of the particle to form a polymeric shell as shown in Figure 1.2. The hydrophobic drug can be encapsulated within the polymeric shell. The order of assembly for these microparticles is different from the tradition reverse order of assembly for many nanomedical systems. Many systems are manufactured from the core to the outermost shell in the reverse order that the layers are actually used within the body. Due to the templating method used, the order of synthesis is a layer of the polymeric shell followed by a layer of the hydrophobic drug then a final layer of the polymeric shell. The assembly ends with the addition of the collagen binding peptide.

4.1.2 PVA as a Template

As described in the introduction, gelatin was the suggested template to use during synthesis of the microparticles [1]. Gelatin was used for the initial experiments of

microparticle synthesis, however no fluorescence was seen after attempting to conjugate thiol-terminated fluorescent peptide to surface maleimide groups on the template. This suggested that functionalizable maleimides were not present on the surface of the microparticles. It was hypothesized that the maleimide was reacting with the gelatin, since gelatin contains lysine which contains a free amine group [2]. During the time of solvent evaporation or when the gelatin was being dissolved away from the formed particles, it is possible that the maleimides were reacting with the lysine in the gelatin. PVA has been used to synthesis particles in other studies and does not contain groups that are reactive with maleimide. For this reason, PVA template was chosen to prevent the unwanted maleimide reaction with the template. Microparticles synthesized using a PVA template allowed for successful attachment of FITC-SILY to the maleimide on the surface of the microparticles

4.1.3 Multilayered Potential

The three layers of polymers that compose these microparticles allow for a unique drug delivery system. The unique layers of the microparticle allow for different functions to be present within one delivery system. The hydrophilic shell prevents agglomeration of the microparticles and enhances the interaction with the body's aqueous environment. The hydrophobic polymer core allows for the incorporation of hydrophobic drugs in future experiments. The designed procedure can be modified to include more layers through decreasing the polymer concentrations. The potential for adapting this procedure is limitless and can include layers to enhance imaging of the microparticles, pH specificity, or multiple drug layers.

4.1.4 Incorporating Hydrophilic Polymers

Previous experiments using the Park templating method used the hydrophobic polymer PLGA and/or PLGA-drug combinations for microparticle synthesis [1, 3]. So, the Park templating method needed to be adapted for synthesizing microparticles with a hydrophilic PEG shell. PLGA with drug combinations was shown to maintain its structure as a cylinder or as alphabet letters and was found not to diffuse into the template [1]. Diffusion into the hydrophilic template was prevented because a hydrophobic nonpolar solution was used. A solely hydrophilic solution would be miscible with the template and diffuse into the template. So, the PLGA-PEG-Mal provides the hydrophobic PLGA and the hydrophilic PEG that when the solvent is slowly evaporated from the polymer solution, the PEG interacts with the PVA and the PLGA is exposed to the air thus forming a core shell in the process. Overall, the hydrophilic shell will prevent aggregation in the body and specifically in the blood system.

4.1.5 Comparison to Other Hydrogel Synthesis Procedures

The Park templating method varies greatly from other methods of microparticle synthesis such as oil immersion techniques. However, the molding technique for microparticle synthesis represents a similar synthesis method. For the molding technique by Yung and Li, a polymer is poured into a mold of any shape and size. A gelling agent or temperature change causes the polymer microparticle to gel, and the microparticles can be removed from the mold [4]. This molding techniques has similar benefits to the templating method in that uniform particles form, procedure is simple and inexpensive, and there is a potential for scalable fabrication [5]. Yung and Li successfully attached a

reactive fluorescein to the microparticles using chitosan's abundant amine groups showing the microparticles' targeting ligand conjugation potential [5]. This molding technique shows an example of functionalizable microparticle composed of PEG using a hydrogel synthesis procedure. Future studies could analyze the use of chitosan-PEG use with the Park templating method.

4.2 Evaluation of Microparticles

4.2.1 Validation of FITC–SILY Attachment to Microparticle Surface

The hypothesis of this validation experiment was that FITC-SILY would attach to the surface of the microparticles through a reaction with the maleimide and the cysteine in the SILY. The results of the validation of FITC-SILY attachment show microparticles with a distinct fluorescent FITC-SILY shell around a non-florescent core. The non-florescent core represents the non-reactive PLA and PLGA. The FITC-SILY florescent shell indicates that the SILY is reacting with the free maleimides present on the surface of the microparticles. Figure 3.3 does show minimal fluorescence on the negative control microparticles, however compared to the experimental microparticles there is significantly more fluorescence. This minimal fluorescence seen can be attributed to some non-specific interactions. The confirmed peptide binding capabilities show that this microparticle system has the potential to be a platform for attachment of any peptide containing a free thiol or amine.

4.2.2 Quantification of FITC-SILY Attachment to Microparticles

The hypothesis of this quantification experiment was that there would be a statically higher concentration of attached FITC-SILY for the experimental microparticles (Mal-PEG-PLGA) in comparison to the negative control particles (Methyl-PEG-PLGA). The FITC-SILY concentration of 2mg/mL that was used in the Validation of Functionalizable Surface Maleimide experiment was shown to incompletely dissolve in PBS leaving free unattached dye that then contaminated the samples, specifically the negative control microparticles. So this experiment analyzed the

optimal FITC-SILY concentration and preparation method for attachment and quantification. Different concentrations of FITC-SILY were prepared at concentrations between 0.0625 and 0.0078 mg/mL because these concentrations left the least amount of undissolved FITC-SILY. The undissolved FITC-SILY can be attributed to insufficient mixing or a high concentration of FITC-SILY. Various chemicals can be used to enhance dissolving of the FITC-SILY.

Both, a 1% bovine serum albumin (BSA) and 0.02% Tween 20 were independently used to block non-specific interactions between the FITC-SILY and the negative control microparticles. Also in attempt to reduce the hydrophobic interaction between the PLGA and the FITC, the molecular weight of the PLGA was reduced while increasing the molecular weight of the PEG. So, a methyl-PEG-PLGA with a molecular weight of 5,000:4,000 Da was purchased from Akina Inc and used to form the polymer shell of the negative control microparticles. However, the results of these experiments were not statistically different but brought this research one step closer to quantifying the attachment of the FITC-SILY.

The most significant problem was that undissolved FITC-SILY was still present in the samples. In order to address this issue, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) was added to the FITC-SILY solution to increase the solubility in PBS and the excess undissolved dye was removed with centrifugation prior to reaction with the microparticles. This experiment produced significantly different FITC-SILY concentrations between the experimental and negative control microparticles. A concentration of 0.0156mg/mL FITC-SILY with TCEP was determined to be the ideal method for the synthesis of microparticles for quantifying the FITC-SILY. The validation

of FITC-SILY attachment experiment further confirmed the successful attachment of FITC-SILY to the maleimides present on the surface of the microparticles.

4.2.3 Validation of Microparticles' Collagen Binding Capabilities

The hypothesis of the validation of collagen binding experiment was that the SILY-MAL-PEG-PLGA microparticles would have significantly greater binding to type I collagen compared to the negative control (Me-PEG-PLGA) microparticles. The SILY was prepared at the ideal concentration of 0.0156mg/mL which was determined during the quantification experiments. The amount of microparticles bound to the type I collagen plate was significantly greater than the negative control microparticles. These results show that this microparticle system has significant clinical application in that it can bind specifically to type I collagen and potentially target atherosclerotic regions of blood vessels.

4.3 Microparticle Degradation Observations

Upon synthesis of initial microparticles, rapid degradation of the microparticles was observed. These initial microparticles contained the layers of PEG and PLGA without the conjugation of the collagen-binding peptide. The degradation of these particles was attributed to the presence of PEG on the surface of the microparticles and the inefficient packing of molecules within the microparticle. PEG is known to degrade at elevated temperature in the presence of oxygen and water through oxidation [6]. The solution for this problem was the incorporation of a dense core. For this reason, PLA was the chosen polymer based on biocompatibility and common use in drug delivery systems. The PLA forms a dense core by adding uniformity and tight packing to the core. Through the synthesis process, a 20% w/v PLA was the highest concentration of polymer that did not form a scum layer. A scum layer is produced when a polymer layer forms on top of the PVA template and adjoins all the microparticles together. This is highly undesirable and is prevented by ensuring that the polymer is evenly spread before the solvent evaporates. To further prevent degradation, a 5% w/v PLGA-PEG-Mal was chosen as the lowest concentration to provide polymer to each well with minimizing the disorganization of polymers which causes rapid degradation.

4.4 Conclusions and Future Research

The designed microparticle drug delivery system has the potential to target any type I collagen in the body, specifically atherosclerotic vessels. Further research should be conducted to synthesis 5μm microparticles using the currently available PDMS template by Akina, Inc. This size particle would allow for clearance of the venous system without occlusion [7]. Also, continued research should be performed with in-vitro testing to validate the biocompatibility and cytotoxicity of the microparticles with targeted cells. In-vivo testing should also be performed to analyze the biodistribution of the microparticles within the body. However, the expected biodistribution results should show targeting to type I collagen after the reduction of the particles' size to 5μm.

Impurities present in Figures 3.2-3.3 are shown among the microparticles. These impurities may be present due to excess PVA that was not properly dissolved and removed. Future studies should be performed to optimize the procedure in order to prevent these impurities potentially by increasing the time or temperature during PVA dissolving.

Further research should be concluded to encapsulate drug within the PLGA core. Ideal drugs for this drug delivery system are drugs that require targeting to particular tissues or dugs with low solubility and low oral bioavailability. Hydrophobic drugs are known for their poor solubility in water. These drugs can be categorized as follows [8]:

- Slightly soluble: 1-10mg/mL
- Very slightly soluble: 0.1-1mg/mL
- Practically insoluble: <0.1mg/mL

Examples of PLGA based microparticles that have entered the market include: octreotide acetate, leuprolide acetate, triptorelin acetate, triptorelin pamoate, triptorelin, ect. [9]. Park et al. used the templating method to incorporate the hydrophobic drugs: felodipine, risperidone, progesterone, and paclitaxel [3]. Many different researchers have incorporated hydrophobic drugs into PLGA cores, but studies have also determined methods to incorporate hydrophilic drugs within the core. Gaignaux et al encapsulated anti-inflammatory and hydrophilic drug, Clonidine, into PLGA microparticles with the use of large aqueous phases to form the second water-in-oil-in-water (w/o/w) emulsion [10]. Further research including: scaling down the size of the microparticles, purifying the synthesized microparticles, and incorporating drug in the microparticle core will increase the clinical impact of this microparticle system.

4.5 References

[1] Acharya, G., Shin, C.S., McDermott, M., Himanshu, M., Park, H., Kwon, I.C., and Park, K., The Hydrogel Template Method for Fabrication of Homogeneous Nano/microparticles. *Journal of Controlled Release,* 2010. **141**(3): 314-319.

[2] Raja Mohd Hafidz, R. N., Yaakob, C.M., Amin, I., and Noorfaizan, A., Chemical and Functional Properties of Bovine and Porcine Skin Gelatin. *International Food Research Journal,* 2011. **18**: p. 813-817.

[3] Acharya, G., Shin, C.S., Vedantham, K., McDermott, M., Rish, T., Hansen, K., Fu, Y., and Park, K., A Study of Drug Release from Homogeneous PLGA Microstructures. *Journal of Controlled Release*, 2010. **146**(2): p. 201-206.

[4] McClement, D.J., Nanoparticle- and Microparticle-Based Delivery Systems: Encapsulation, Protection and Release of Active Compounds. Science, 2014. p. 312.

[5] Jung, S. and Yi, H., Fabrication of Chitosan-Poly(ethylene glycol) Hybrid Hydrogel Microparticles via Replica Molding and Its Application toward Conjugation of Biomolecules. *Langmuir*, 2012. **28**(49) 17061-17070.

[6] Glastrup, J. 1995. Degradation of Polyethylene Glycol. A Study of the Reaction Mechanism in a Model Molecule: Tetaethylene Glycol.Polymer Degradation and Stability. *Polymer Degradation and Stability,* 1996. **52**(3): 217-222.

[7] Kohane D.S., Microparticles and Nanoparticles for Drug Delivery. *Biotechnology and Bioengineering*, 2007. **96**(2): p. 203-209.

[8] Li, J., Feng, L., Fan, L., Zha, Y., Guo, L., Zhang, Q., Chen, J., Pang, Z., Wang, Y., Jiang, X., Yang, V., and Wen, L., Targeting the brain with PEG-PLGA nanoparticle modified with phage-displayed peptides. *Biomaterials,* 2011. **32**(21): p. 4943-4950.

[9] Wischke, C. and Schwendeman, S. P., Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *International Journal of Pharmaceutics*, 2008. **364**(2) 298-327.

[10] Gaignaux, A., Reeff, J., Siepmann, F., Siepmann, J., De Vriese, C., Goole, J., and Amighi, K., Development and Evaluation of Sustained-Release Cloidine-Loaded PLGA Microparticles. *International Journal of Pharmaceutics,* 2012. **437**(1-2): p. 20-28.